Formation and structure of alkali metal, thallium, silver and alkaline-earth cation complexes with the ionophore lasalocid free acid form in methanol from NMR experiments

Mostafa Mimouni,[a] Rachid Lyazghi[b] and Jean Juillard*[,b]

[a] *Laboratoire de Chimie Analytique Appliquée, Faculté des Sciences Mohamed I°, route Sidi Maafa, BP 524, Oujda, Morocco*
[b] *Laboratoire de Thermodynamique des Solutions et des Polymères (CNRS UPRES A 6003), Université Blaise Pascal, 63177 Aubière cédex, France*

Variations in the ^{1}H and ^{13}C chemical shifts of lasalocid in methanol as a function of the concentration of added metal cations allow simultaneous access to the formation constants of the corresponding acid complexes and to the NMR spectra of the species formed. It is shown that the acid complexes present a marked structural analogy to the neutral complexes, resulting from the interaction of lasalocid anion with metal cations, in the same solvent. Assuming a two-step process involving first the formation of the acidic complex and then of the neutral one, the selectivity of the ionophore can mainly be ascribed to the first step, the formation of a complex between the cation and the ionophore acid molecule.

Formation et structure dans le méthanol des complexes de l'ionophore lasalocide acide avec les cations alcalins et alcalino-terreux, argent et thallium: étude RMN. L'étude des variations des déplacements chimiques ^{1}H et ^{13}C du lasalocide en fonction de la concentration en cation métallique permet d'accéder simultanément aux constantes de formation des complexes acides correspondants et à leurs spectres de RMN. On montre alors que ces complexes acides présentent sur le plan structural une analogie certaine avec les complexes formés, dans le même milieu, par l'anion lasalocide et les cations métalliques. La formation de ces complexes neutres du lasalocide, seuls susceptibles de migrer au travers des membranes biologiques, apparaît être un processus en deux étapes: la formation du complexe acide et sa déprotonation; la sélectivité de l'ionophore pour les divers cations est largement déterminée lors de la première étape.

Lasalocid (Fig. 1) is a bacterial ionophore, obtained from *Streptomyces lasaliensis* strains, that is able to carry cations across natural and artificial membranes through a proton-cation antiport process. In the mechanism of this transport of M^+ cations by such ionophores HA, it is more than probable that, though the active species is MA, the acidic complex HMA^+ occurs as an intermediary species in the kinetic process. These species are not unstable transition complexes, but have been proved to be stable in both protic and aprotic organic solvents.[1–4] However, their formation constants are much lower than those of the corresponding MA salts. Formation of acid complexes between these ionophores and divalent cations is also probable, though it has not often been considered.[5] Their corresponding formation constants must be weak. Though the active process in which ionophores are engaged occur at the interface of the aqueous/lipid phases, they have been frequently studied in methanol, a solvent in which all the species (ionophores, ionophore metal complexes and simple metal salts) are soluble. For lasalocid in methanol, the formation constant of the MA and MA_2 complexes are now well-established.[2,6–8] Structural aspects in solution concerning these complex salts were also discussed from NMR and computational data.[9] However, only limited data is reported on HMA^+ formation[2,8] and little is known about the structure of these species.

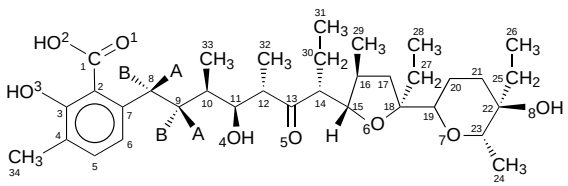

Fig. 1 Lasalocid formula showing carbon and oxygen numbering scheme

The work reported here was carried out to obtain both the formation constants and structures of lasalocid acid complexes of monovalent and divalent metal cations. For this purpose ^{13}C and ^{1}H NMR was chosen. A nonambiguous assignment of the ^{13}C and ^{1}H chemical shifts of lasalocid acid in methanol has already been reported[10] and the structural features of this species were recently discussed in relation to its computational modeling.[11] This was thus a good starting point for this study.

Experimental

Chemicals

Unlike that obtained some years ago, the lasalocid prepared as previously[12] using the currently commercialized sodium salt (Sigma) was impure (<98%). These new samples were thus purified by chromatography on a silica column (Amicon 20 Å 70–200 μm) using a mixture (90 + 10) of cyclohexane and ethyl acetate. Each eluted fraction collected was tested by thin layer chromatography. This showed that most of the impurities migrated in the first fractions; thereafter the solvent balance in the mixture was changed to (80 + 20). As the column was appreciably acidic, what was obtained after evaporating the main central fractions was a mixture of lasalocid acid and lasalocid sodium salt. This mixture was dissolved in chloroform. This phase was treated with an aqueous 0.5 mol L^{-1} nitric acid solution. The rest of the preparation

was as usual.[12] It yielded pure samples of lasalocid acid; typical potentiometric titrations showed a 99.9% lasalocid content.

The inorganic metal salts used were of the best commercially available grades (Merck Suprapur, Prolabo RP Normapur, *etc.*). RbSCN and CsSCN were prepared from $Ba(SCN)_2$ (Ventron XTL, 99.9%) and Rb_2SO_4 (Strem Chemicals NATL) or Cs_2SO_4 (Ventron XTL, 99.9%) in aqueous solution according to a procedure[13] involving the reaction

$$Ba(SCN)_2 + Rb_2(SO_4) \rightarrow 2RbSCN + BaSO_4$$

The salts thus prepared were titrated potentiometrically using reduction of the thiocyanate by iodate in acidic medium.[14]

CD_3OD was a Merck Uvasol (99.8%) product. A 12 N D_2SO_4 solution in D_2O was obtained from Spectrometric Spin Technique (SST, 99.00%).

NMR data

^{13}C and ^1H NMR data were acquired as previously described[10] using either Bruker MSL 300 or AC 400 spectrometers. Chemical shifts are referred to tetramethylsilane. ^7Li and ^{23}Na experiments were performed using the 300 MHz apparatus equipped with the multinuclei probe at 116.644 and 79.35 MHz, respectively, using 10 mm diameter tubes. Shimming and field frequency locking were carried out with D_2O. NMR spectra were accumulated for 4 to 40 min to obtain a good signal-to-noise ratio. The main parameters were as follows: 90 pulses (10 μs), repetition time approx. 0.35 s, 600 to 6000 scans, 5 dB, 2K data.

Methods and Results

Acquiring and processing ^{13}C and ^1H spectra

The method used consisted in adding to a solution of lasalocid HA, of analytical concentration c_A^* in CD_3OD, increasing amounts of the inorganic salts MX or MX_2 (salt analytical concentration c_M^*). Both ^1H and ^{13}C, ^1H decoupled spectra were recorded at each step. In some cases, to repress any dissociation of lasalocid, a small amount of a D_2SO_4 solution in CD_3OD was added to the solvent to obtain a 10^{-5} mol L^{-1} solution of D_2SO_4. However, no appreciable changes were observed in the spectra in the presence or absence of D_2SO_4. Assignments of ^1H and ^{13}C resonances in 1D spectra were obtained by analogy with those of the lasalocid free acid. They were checked through the analysis of the contour plots of the ^1H-^1H correlations (COSY 90°) obtained from one definite value of the c_M^*/c_A^* ratio. Some inversions were noted; they were correctly assigned by considering the corresponding *J*-modulated spin echo spectra. The 1D spectra were in all cases well resolved, significant enlargement or splitting of the signals not being observed. However, for most of the signals a smooth and monotonous variation of their location was observed as a function of the salt concentration. The spectra of the species MA and MA^+, which correspond to the displacement of the proton by M^+ or M^{2+}, are known.[9,10] From the examination of the ^{13}C signal location change it can be ascertained that such species were not, in any case, formed to any extent under our experimental conditions. The variations of the ^1H and ^{13}C chemical shifts were thus attributed to interactions between the metal cation M^+ or M^{2+} and the ionophore acid HA, exchanges between free acid and metal complexes thus being rapid on the NMR timescale. Concentrations c_A^* used in the experiments strongly depended on the solubility of the accessible inorganic salts MX or MX_2. c_A^* generally ranged between 0.05 and 0.1 mol L^{-1}, except in the case of Ag^+ and Tl^+ for which concentrations in the 10^{-2} mol L^{-1} range were used, the acquisition time being in this case greatly increased.

Though the formation between metal M^{z+} and ionophore HA of various successive complexes $M(HA)_n^{z+}$ could have been envisaged *a priori*, as a first approximation, only the species MHA^{z+} was considered ($z = 1$ or 2). The reaction involved was thus:

$$HA + M^+ \rightarrow HMA^+ \quad (1)$$

or

$$HA + M^{2+} \rightarrow HMA^{2+} \quad (1')$$

The two formation constants are:

$$K_1 = [HMA]y_1^{\pm}/[M^+][HA]y_1^{\pm} \quad (2)$$

$$K_1' = [HMA]y_2^{\pm}/[M^{2+}][HA]y_2^{\pm} \quad (2')$$

in which $y_1^{\pm}$ and $y_2^{\pm}$ stand for the mean molar activity coefficients for monovalent or divalent species and the species in brackets stand for their concentrations. As these reactions are isoelectric, these coefficients should vanish in the expression of the equilibrium constants. Mass balance equations are:

$$c_A^* = [HA] + [HMA^{z+}] \quad \text{and} \quad c_M^* = [M^{z+}] + [HMA^{z+}] \quad (3)$$

The observed chemical shift for a given proton or a given carbon δ is related to its chemical shift in the free acid δ_{HA} and in the complex δ_{HMA} according to:

$$c_A^*\delta = [HA]\delta_{HA} + [HMA^{z+}]\delta_{HMA} \quad (4)$$

which can be rearranged as

$$\delta = \delta_{HA} + [HMA^{z+}](\delta_{HMA} - \delta_{HA})/c_A^* \quad (5)$$

Data corresponding to one signal and eqn. (2) to (4) enable us to determine K_1 or K_1' and δ_{HMA} together. Practically, we searched for a value of K_1 or K_1' that best linearized the curve:

$$\delta = f(r) \quad \text{with} \quad r = [HMA^{z+}]/c_A^*$$

Examples of the search are given in Fig. 2. Extrapolation to $r = 1$ thus yielded the value of δ_{HMA}. Calculations were refined using a least-squares treatment. This procedure thus gives access to the spectra of HMA^{z+} species that could not, owing to the weakness of the equilibrium constants, be experimentally attained. Such calculations were only performed on the signals that presented the most significant variations as a function of the cation analytical concentration. The K_1 value retained for each carbon corresponded to the best linear fit of eqn. (5) using a least-squares treatment. The accuracy reported in Table 1 includes both the confidence degree of this value for

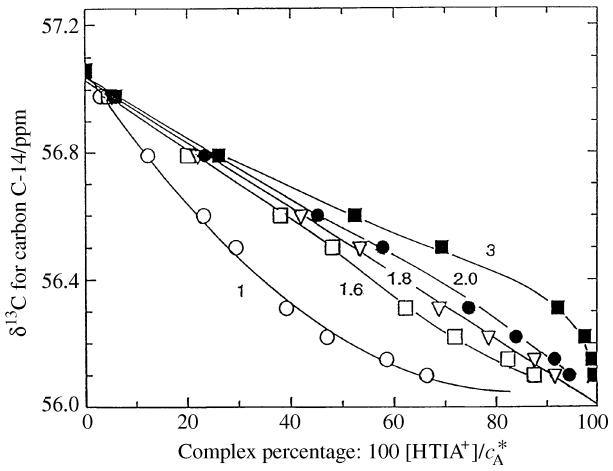

Fig. 2 Variation of ^{13}C chemical shifts with calculated percentage of the acid complex $HTlA^+$, assuming values of the equilibrium constant given on the corresponding curves. Log $K_1 = 1.8$ corresponds to a reasonable fit of eqn. (5)

Table 1 Formation constants of acid complexes of lasalocid with various cations in CD_3OD (temperature $\approx 20\,^{\circ}C$, molar scale of concentrations). Ionophore analytical concentration c_A^*, range of metal inorganic salt analytical concentration c_M^*, calculated constant K_1 or K_1' and estimated accuracy

Nucleus	Salt	c_A^*/mol L^{-1}	c_M^*/mol L^{-1}	Species	log K_1 or K_1'	log K_1 or K_1' from potentiometry
^{13}C	LiCl, $LiClO_4$	0.10	0.11–0.50	$HLiA^+$	0.0 ± 0.2	
^{13}C	NaBr	0.005	0.005–2.80	$HNaA^+$	0.8 ± 0.2	0.7[8]
^{13}C	KI, KPF_6	0.02	0.02–0.84	HKA^+	1.8 ± 0.2	1.3[8]
^{13}C	RbSCN	0.10	0.01–0.90	$HRbA^+$	1.2 ± 0.4	1.6[8]
^{13}C	CsSCN	0.07	0.01–0.16	$HCsA^+$	2.0 ± 0.4	
^{13}C	$AgNO_3$	0.01–0.05	0.03–0.09	$HAgA^+$	2.0 ± 0.3	2.0
^{13}C	$TlPF_6$	0.1	0.003–0.018	$HTlA^+$	1.8 ± 0.2	
^{13}C	$Mg(ClO_4)_2$	0.07	0.08–0.44	$HMgA^{2+}$	-1.0 ± 0.5	
^{13}C	$CaCl_2$, $Ca(ClO_4)_2$	0.05	0.05–1.29	$HCaA^{2+}$	0.0 ± 0.3	
^{13}C	$SrCl_2$	0.06	0.05–1.41	$HSrA^{2+}$	0.5 ± 0.2	
^{13}C	$BaCl_2$, $Ba(ClO_4)_2$	0.07	0.04–0.341	$HBaA^{2+}$	1.8 ± 0.2	
^{7}Li	$LiClO_4$	0.014–0.190	0.0046	$HLiA^+$	not sensitive	
^{23}Na	$NaClO_4$	0.006–0.2160	0.0040	$HNaA^+$	0.6 ± 0.4	

each carbon and the dispersion from the mean value obtained for the various carbons.

Satisfactory results were thus obtained assuming the formation of 1 : 1 complexes alone. Attempts to process data considering instead the formation of complexes involving two molecules of ionophore acid, $H_2MA_2^{2+}$, failed. A linear fit of the corresponding equation [analogous to eqn. (5)] could never be obtained, whatever the value taken for the formation constant of this species. Hence preponderant formation of 1 : 1 complexes is evident. Formation of 2 : 1 complexes, if any, can only be slight.

Using the constant thus obtained for all the ^{13}C chemical shifts and the readily accessible ^{1}H chemical shifts then enabled us to determine δ_{HMA} for all the signals, as shown in the example given in Fig. 3. Formation constants K_1 and K_1' of the acid complexes thus obtained are reported in Table 1, and values of $(\delta_{HMA}{}^{z+} - \delta_{HA})$ are given in Fig. 4 and 5 for the various cations studied, except for magnesium for which they were considered to be too inaccurate to report. The precision reported in Table 1 for the formation constants may appear rather poor. This precision is affected by the difficulty of preparing solutions of accurate concentration in such small volumes (0.5 ml) for a volatile deuterated solvent, the possibly incomplete dissociation of some of the salt used in this solvent, the water content, which can become appreciable as the salt concentration increases, and the polarization induced by the high electrolyte concentration. All these disadvantages, which are inherent to this method, make it only a moderately accurate method; its great advantage is to give access concurrently to structural features.

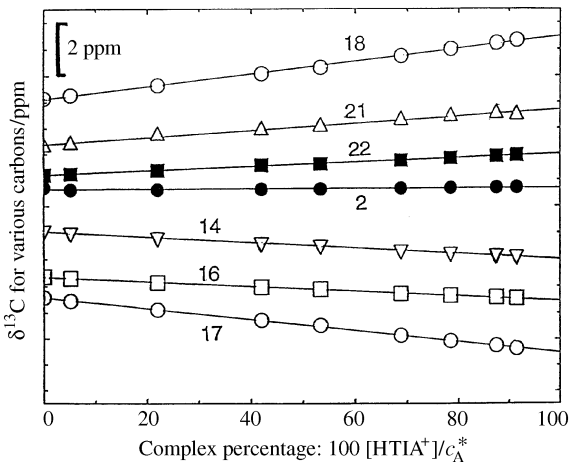

Fig. 3 Variation of the ^{13}C chemical shift of some carbon atoms *versus* the percentage of $HTlA^+$ formed using log $K_1 = 1.8$

Acquiring and processing ^{7}Li and ^{23}Na data

Formation equilibria of HMA^+ species were also studied using ^{7}Li and ^{23}Na NMR and recording these spectra. The inorganic salt analytical concentration was this time kept constant ($c_M^* = 4 \times 10^{-3}$ mol L^{-1}) and successive amounts of lasalocid acid were added (ionophore analytical concentration c_A^*). Under these conditions the system is governed by eqn. (2), (3) and (6).

$$c_M^* \delta = [M^+]\delta_M + [HMA^+]\delta_{HMA} \quad (6)$$

In practice, the initial signal observed was taken as the origin ($\delta_M = 0$). No line broadening was observed. In the case of lithium ion, chemical shift variations were too weak (a maximum of 0.04 ppm for a 1 : 7 lithium : lasalocid ratio) to allow a correct calculation of the constant K_1. In the case of

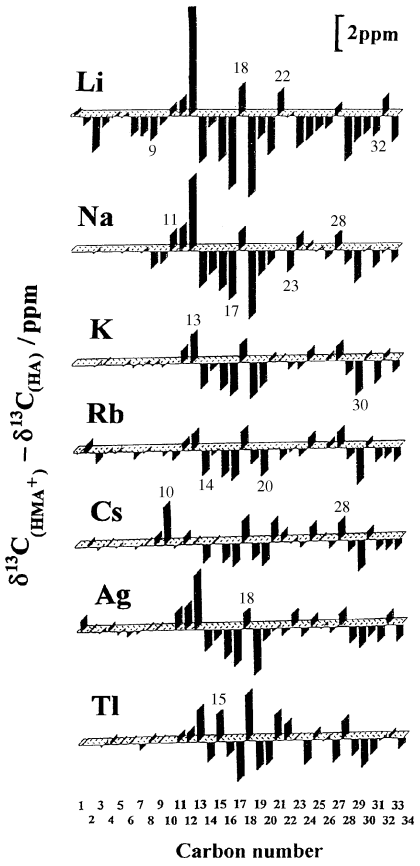

Fig. 4 Variation of the ^{13}C chemical shift in methanol for all carbon atoms in going from free lasalocid to the acid monovalent cation complex. $\delta\,^{13}C_{(HMA^+)} - \delta\,^{13}C_{(HA)} = f$(carbon number)

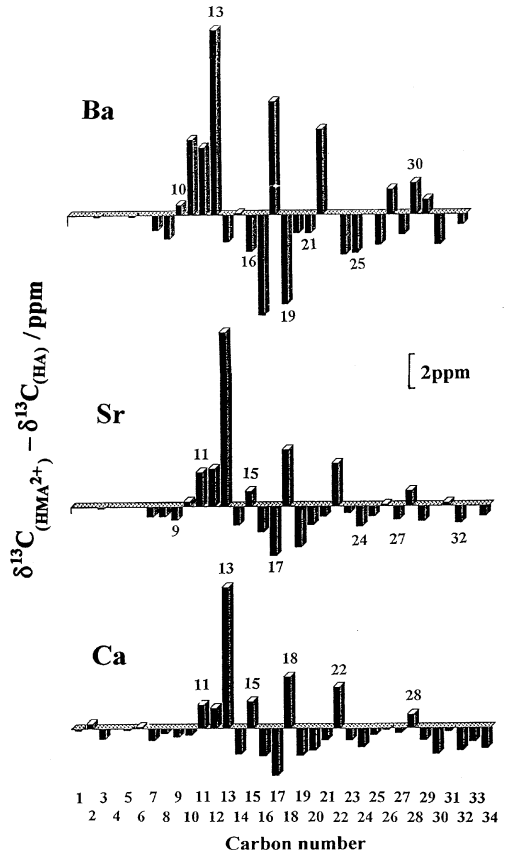

Fig. 5 Variation of the ^{13}C chemical shift in methanol for all carbon atoms in going from free lasalocid to the acid divalent cation complex. $\delta\,^{13}C_{(HMA^{2+})} - \delta\,^{13}C_{(HA)} = f$(carbon number)

sodium ion, chemical shift variations were appreciable. The value obtained for the formation constant of the complex, also reported in Table 1, agrees well with the value obtained from the ^{13}C data.

Comparing with previous data

Formation constants K_1 were previously obtained for some cations (Na^+, K^+ and Rb^+) using two indicative electrode (for M^+ and H^+) potentiometric titrations.[8] These values are gathered in the last column of Table 1. A new result for silver ion, obtained using the same procedure, is also included. As compared to the present NMR determinations the agreement is good for sodium and silver and acceptable for rubidium. Concerning potassium, the potentiometric value (log K_1 = 1.3) appears to be low, not only as compared to the present NMR value, 1.8, but also as compared to the value obtained by Degani and Friedman[2] from circular dichroïsm measurements, log $K_1 = 2.2$.

Discussion

Formation of lasalocid acid and lasalocid anion metal cation complexes

As suspected, the formation constants K_1 corresponding to reactions 1 (or 1′) were considerably weaker than the constants K_2 or K'_2 corresponding to reactions 7 (or 7′):

$$A^- + M^+ \rightarrow MA \quad (7)$$

$$A^- + M^{2+} \rightarrow MA^+ \quad (7')$$

This is shown for the various cations in Fig. 6 and 7. Values used for K_2 are from the literature[7,8] except for thallium and silver, which were lacking. The formation constants of the TlA salt in methanol at 25 °C were determined according to the

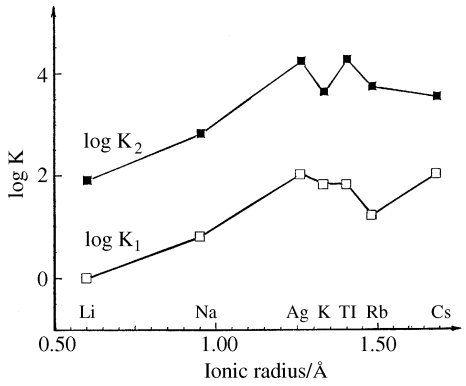

Fig. 6 Formation constants in methanol of (□) acid complexes [reaction (1)] at ≈20 °C and (■) neutral complexes [reaction (7)] at 25.0 °C of the monovalent cations with lasalocid ionophore

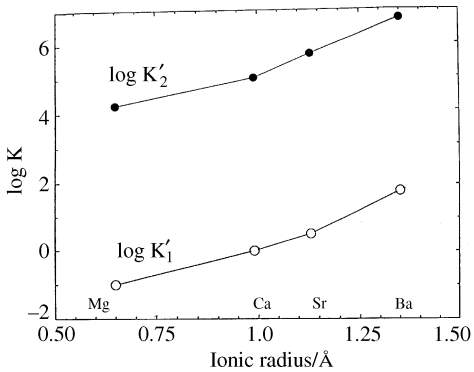

Fig. 7 Formation constants in methanol of (○) acid complexes [reaction (1′)] at ≈20 °C and (●) neutral complexes [reaction (7′)] at 25.0 °C of the divalent cations with lasalocid ionophore

method and procedures described for the alkali metal salts.[8] A value of log $K_2 = 4.2 \pm 0.1$ was obtained. Using the same procedure in the case of silver led to a more complex situation; formation of species other than AgA has to be taken into account. Though this problem is not yet entirely solved, a provisional value of log $K_2 = 4.2$ has been used here in Fig. 6. A value of log $K_1 = 2$ was obtained from the acid part of potentiometric titration curves; this value is in close agreement with that independently obtained using ^{13}C NMR.

Comparing in Fig. 6 and 7 the formation of the neutral and acid complexes it can be seen that analogous trends occur in the variation of the equilibrium constants as a function of the size of the cation concerned. Such an analogous trend in the variation of the formation constant of MA and HMA^+ with the nature of the cation was previously observed in the case of monensin in acetonitrile[15] and thus seems to be usual for carboxylic ionophores. Here for monovalent cations, the two formation constants differ only by two log units, whereas for divalent cations they differ by about five log units. Other things being equal, the formation of MA^+ predominates over the formation of HMA^{2+} at lower pH than that at which the formation of MA predominates over the formation of HMA^+.

Structure of acid complexes of lasalocid in methanol

We shall now consider the patterns in Fig. 4 and 5, which show for each carbon the variation of its chemical shift in going from the free acid to the acid in the HMA^{z+} complex. As already emphasized,[9] variation through cation complexation of the ^{13}C chemical shift of a given carbon of a ligand can be attributed to three main effects: interaction of the metal ion with a neighbouring oxygen atom, conformational changes and changes in the interactions with the neighboring solvent molecules.

The first of these effects would result in a deshielding of the carbons located near coordinating oxygens and thus in a downfield shift of their resonance frequencies. Such an effect can sometimes be counterbalanced by the other effects, which are mainly related to structural changes associated with cation complexation. Nevertheless, a net positive variation of such a ^{13}C chemical shift can generally be taken as a strong indication of the involvement of the vicinal oxygen in the cation coordination. Examination of Fig. 4 thus suggests, from the effects observed on C-11, C-13, C-15, C-18 and C-22, that O-4, O-5, O-6 and O-8 are involved in the coordination of the alkaline-earth cations, the strongest involvement being that of the ketonic oxygen. The intensity of this interaction decreases as the size of the cation increases. Considering the effects on the other carbons from C-10 to C-24, particularly on those belonging to the rings, the chemical shift variations of which could mainly be ascribed to conformational changes, it is suggested that the deformation of the ionophore backbone is less and less marked as the size of the cation increases. However, the most striking fact observed in Fig. 4 is that the chemical shifts of carbons C-1 to C-10 are practically unaffected by cation complexation. This means that the conformation of the salicylic moiety of the ionophore molecule does not appreciably vary, and that the carboxylic function is not involved in the coordination of the cation.

Interaction strengths of cations with oxygen coordinating sites decrease with their charge. The effects in the chemical shift variations resulting from the coordination are less marked for monovalent than for divalent cations. However, analogous trends are observed, though a wider variability as a function of the size of the cation occurs. From NMR data reported in Fig. 5 it appears that in most cases, chemical shift variations are negligible or very weak in the area C-1 to C-10, which shows the conformational stability of this area and indicates the non-implication of the head of the molecule in the coordination of the cation. In the case of lithium, for which perturbations apparently occur in this area, it must first be emphasized that the results obtained are less accurate than those for other monovalent cations owing to the weakness of its complex formation constant, which implies less sure extrapolations of the chemical shifts. This also caused us to carry out measurements in highly concentrated solutions of lithium salts; the disturbance observed in the area C-1 to C-10 could then well result from a restriction of the solvation of this salicylic moiety owing to the strong solvation of Li^+. Hence, it is not possible to draw conclusions on the involvement of the carboxylic function in the coordination of lithium ion, unlike the other cations for which such coordination can be ruled out.

Concerning the other coordinating sites, the same oxygens are potentially involved. As shown by the variations in the C-13 chemical shifts, if lithium or sodium cations strongly interact with O-5, this interaction regularly decreases with the size of the cation, from sodium to cesium. As in the case of divalent cations, variations observed on the carbons not bound to oxygens follow the same trends and the amplitude of these variations decreases as the size of the cation increases, which shows that the conformation of the acid ligand is less disturbed by large cations than by small cations like lithium or sodium. The ligand cavity may adapt to wrap smaller cations better.

The patterns presented in Fig. 4 and 5 can be usefully compared with analogous patterns reported previously for the variation of the ^{13}C chemical shifts from the lasalocid anion A^- to the lasalocid monovalent cation complex MA or divalent cation complex MA^+ under the same conditions (methanol, room temperature).[9] Concerning the Ca, Sr and Ba 1 : 1 complexes of HA and A^-, a strong analogy between these two sets of data is observed except in the area C-1 to C-11. Taking apart this area, the analogy is also appreciable in the case of the alkali metal cation complexes (a small shift of the carbon numbering scale in Fig. 8 of ref. 9*b* makes such a comparison a bit difficult but, once this shift is corrected, the adequacy between these results and the present data is satisfying). Thus, it can be concluded that much of the structure of the lasalocid complexes (conformation of the ligand, location of the cation and interaction with the coordinating sites) is acquired in the acidic complexes; differences with the corresponding anionic complexes concern mainly the carboxylic arm and its hinge with the main backbone. Most of the conclusions concerning the structures of these anionic complexes and coming from NMR and computational experiments can also be applied to these acidic complexes.

Thermodynamics and structural aspects of successive formation of acidic and neutral cation complexes of lasalocid

It was shown that in the translocation of monovalent metal cations by carboxylic ionophores the cation carrier species was most often the salt MA. This implies its formation from the ionophore HA at the first water/membrane interface. It is generally believed that this is a two-step process, involving successive formation first of HMA^+ and then of MA according to:

$$HA + M^+ \rightarrow HMA^+ \quad (1)$$

and

$$HMA^+ \rightarrow MA + H^+ \quad (8)$$

Similarly it can be supposed, for the formation of the 1 : 1 complexes of the divalent cations, that:

$$HA + M^{2+} \rightarrow HMA^{2+} \quad (1')$$

and

$$HMA^{2+} \rightarrow MA^+ + H^+ \quad (8')$$

Many kinetic studies of ionophore-mediated cation transport have been interpreted thus, for example in phospholipid bilayers,[16] across vesicular membranes[17] or in water-organic triphasic systems.[18] In the case of an organic phase like nitrobenzene it was even shown[19] that HMA^+ could be the cation carrier species, but this is not the usual situation. Concerning homogeneous solvent systems, it was also shown that the formation of MA occurs *via* these two steps and the corresponding kinetic parameters have been obtained in some cases.[20] Though there is no experimental evidence concerning lasalocid itself, there is no reason for it to act differently from other ionophores of its family. We will thus consider these two successive reactions, here for lasalocid in methanol, in terms of both free energy and structural changes. Analogous reactions for divalent cations will also be considered.

Accepting that the values reported in Table 1 can be taken as standard formation constants and using the pK_a value of lasalocid already reported,[7] 8.29, we can calculate the Gibbs functions corresponding to reactions (1) and (8) for the various cations. Data thus obtained are given in Table 2. As a matter of fact reaction (8) corresponds to the acidic dissociation of protonic acid HMA^{2+}. Corresponding pK_a are also given in Table 2. Standard Gibbs functions for the first step [reaction (1) or (1′)] are variable according to the cation involved but are all negative except for magnesium. Standard Gibbs functions for the second step [reaction (8) or (8′)] are always large and positive. This reaction is thus unfavorable. MA or MA^+ species can only be formed by raising the pH of the solution.

In addition it must be noted that these standard Gibbs functions for this second step are akin for the various divalent cations and vary only little for the monovalent ones. This would mean that the selectivity of the ionophore for various cations of a given charge would be mainly determined by the

Table 2 Standard Gibbs function for reactions (1) and (8) of the formation of HMA^+ and MA complexes of lasalocid with monovalent cations in methanol (25 °C, molar scale of concentrations).
(1): $HA + M^{z+} \rightarrow HMA^{z+}$
(8): $HMA^{z+} \rightarrow MA^{(z-1)+} + H^+$

Cations	ΔG_1^θ/kJ mol^{-1}	ΔG_8^θ/kJ mol^{-1}	pK_a (HMA^{z+})
Monovalent cations			
Li^+	0	+36	6.3
Na^+	−5	+36	6.3
K^+	−10	+37	6.5
Rb^+	−7	+34	6.0
Cs^+	−11	+39	6.8
Tl^+	−10	+34	6.0
Ag^+	−11	+35	6.1
Divalent cations			
Mg^{2+}	+6	+18	3.1
Ca^{2+}	0	+19	3.3
Sr^{2+}	−3	+18	3.1
Ba^{2+}	−10	+19	3.3

first step, the formation of the acidic complex, the deprotonation of this complex being essentially ruled by the charge of the cation. As shown in Table 2, the pK_a of this complex would be 6.3 ± 0.4 for the monovalent cations and 3.2 ± 0.1 for the divalent cations. From the data on the formation of $LnHA^{3+}$ for parent ionophore monensin and on the formation of LnA^{2+} for lasalocid[21] a pK_a of −3 can be estimated for trivalent lanthanide cations, Ln. This different pK_a value can be usefully compared to the pK_a of the free acid in methanol: 8.3. Increasing the charge of the cation complexed thus greatly facilitates the acidic dissociation of the complex. As noted before there is no strong structural difference between the acidic and anionic complexes of a given cation except for the carboxylic arm. In the acidic complexes, the carboxylic group is not involved in the coordination of the cation but, in the anionic complexes, the carboxylate group is strongly involved in this coordination, at least through one of its oxygens.[9] The strength of this interaction increases as the charge of the cation increases.[9] This effect would then be determinant in the concomitent increase of the acidic complex dissociation. The absence of strong structural changes in the other part of the molecule would explain why the Gibbs function for the deprotonation process of the acidic complexes is of the same order of magnitude for all the cations bearing the same charge.

Conclusion

In the process of formation and then transport across membranes of neutral complexes of carboxylic ionophores with metal cations, the prior formation of acidic complexes must be emphasized. The major part of the structural rearrangement and thus much of the selectivity is gained at this step. The free energy involved in the further deprotonation of this complex mainly depends on the charge of the cation.

References

1 J. G. Hoogerheide and A. I. Popov, *J. Solution Chem.*, 1979, **8**, 83.
2 H. Degani and H. L. Friedman, *Biochemistry*, 1974, **13**, 5022.
3 Y. Pointud, C. Tissier and J. Juillard, *J. Solution Chem.*, 1983, **12**, 473.
4 J. Juillard, Y. Pointud, C. Tissier and G. Jeminet, in *Physical Chemistry of Transmembrane Motion*, ed. G. Spach, Elsevier, Amsterdam, 1983, p. 239.
5 C. K. Vichwanath and K. R. K Easwaran, *J. Chem. Soc., Perkin Trans. 2*, 1985, 65.
6 B. G. Cox, N. Van Truong, J. Rzeszotarska and H. Schneider, *J. Chem. Soc., Faraday Trans. 1*, 1984, **80**, 3275.
7 J. Juillard, C. Tissier and G. Jeminet, *J. Chem. Soc., Faraday Trans. 1*, 1988, **84**, 951.
8 Y. Pointud and J. Juillard, *J. Chem. Soc., Faraday Trans. 1*, 1988, **84**, 959.
9 (*a*) M. Mimouni, P. Malfreyt, R. Lyazghi, M. Palma, Y. Pascal, G. Dauphin and J. Juillard, *J. Chem. Soc., Perkin Trans. 2*, 1995, 1939; (*b*) P. Malfreyt, R. Lyazghi, G. Dauphin, Y. Pascal and J. Juillard, *J. Chem. Soc., Perkin Trans. 2*, 1996, 1971.
10 R. Lyazghi, A. Cuer, G. Dauphin and J. Juillard, *J. Chem. Soc., Perkin Trans. 2*, 1992, **35**, 11.
11 (*a*) P. Malfreyt, Y. Pascal and J. Juillard, *J. Chem. Soc., Perkin Trans. 2*, 1994, 2031; (*b*) P. Malfreyt, Y. Pascal and J. Juillard, *J. Chim. Phys.*, 1996, **93**, 1129; (*c*) P. Malfreyt, M. Mimouni, Y. Pascal and J. Juillard, *J. Chim. Phys.*, 1996, **93**, 1151.
12 L. Woznicka, C. Lhermet, N. Morel-Desrosiers, J-P. Morel and J. Juillard, *J. Chem. Soc., Faraday Trans. 1*, 1989, **85**, 1709.
13 *Inorganic Synthesis*, McGraw-Hill, New York, 1950, vol. 3, p. 24.
14 C. Charlot and R. Gaugin, *Les méthodes d'analyses des réactions en solution*, Masson, Paris, 1951.
15 Y. Pointud, C. Bernard, S. Touzain, L. Astier, B. Sabatier and J. Juillard, *J. Solution Chem.*, 1997, **26**, 479.
16 F. G. Riddell, S. Arumugam, P. J. Brophy, B. G. Cox, M. C. H. Payne and T. J. Scuthon, *J. Am. Chem. Soc.*, 1988, **110**, 739.
17 B. S. Prabhamanda and M. H. Kombrabail, *Biophys. Biochim. Acta*, 1992, **1106**, 171.
18 S. Perrier, University Thesis, Université Blaise Pascal, Clermont-Ferrand, France, 1995, n° DU 682.
19 G. Du, J. Korita, W. Ruth and P. Vanysek, *J. Electroanal. Chem.*, 1983, **159**, 413.
20 B. G. Cox, P. Firman and H. Schneider, *J. Am. Chem. Soc.*, 1985, **107**, 4297.
21 Y. Layec, Mémoire Ingénieur CNAM, Université Blaise Pascal, Clermont-Ferrand, France, 1990.

Received in Orsay, France, 7th April 1977;
revised M/S received, 2nd October 1977; *Paper* 7/08748I